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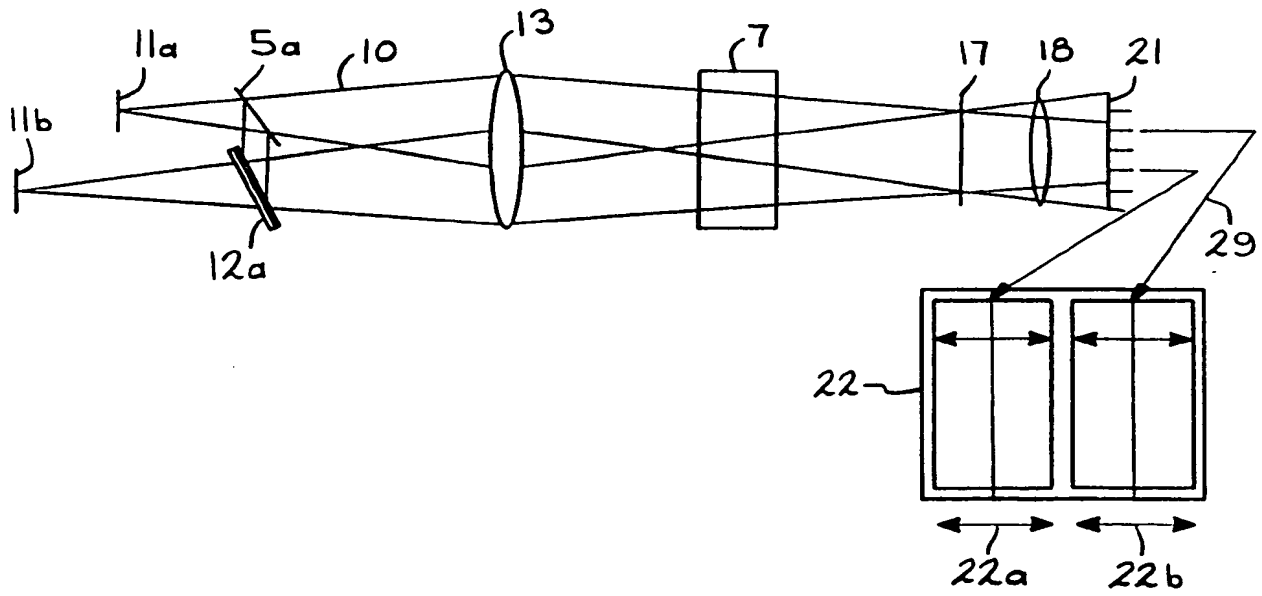

(57) Abstract

A scanning microscope instrument with selected plural optical paths (10, 16) which selectively produce the image of an object (9) with selected light sources (1) and with optical device (5, 6, 7, 12, 14, 15) for directing the light from selected light sources to and from the object by way of a selected optical path for selected conventional and confocal imaging and including direct and indirect observation with selected detection and analytical observation at the final image plane (17).

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MULTIPLE PATH SCANNING MICROSCOPE

Background of the Invention

This invention relates to a scanning optical microscope instrument which provides a selection of optical paths with enhanced contrast of an image of an object by concentration of selected illumination within a certain volume element or set of volume elements of the object and by reducing the amount of light detected from above and below this volume element on or within the object and with selected concurrent observation directly by the human eye and by camera, video and in selected detection modes and times and with confocal observation.

In a conventional optical microscope, the full field of view is illuminated with light from an extended source through a condenser lens. An object within the field of view is imaged within the microscope by the objective lens and can be viewed through an ocular lens. An epi-illumination microscope uses one lens as both the objective and condenser.

In contrast, a confocal microscope illuminates only a small volume element within the field of view using a point or slit source of light. A point or slit detector is used to detect light only from the same volume element. An image of a specimen within the field of view is built up through the relative motion of the specimen with respect to the source and detector by one of several scanning mechanisms.

Minsky (U.S. Patent 3,013,467 (1957)) and later Brakenhoff et al. (J. Microsc. 117: 219-232, 1979) produced confocal images by moving the specimen while maintaining a point source and point detector stationary in the center of the optical axis.

Davidovits and Egger (U.S. Patent 3,643,015 (1972)) designed a confocal microscope whereby a stationary object was scanned by an oscillating objective lens.

Epi-fluorescence illumination uses a single lens as both the objective and condenser. This allows schemes using one single-sided mirror that rotates in two axes (Steltzer, et al. (1988) SPIE 1028, 146-151), or two single-sided mirrors rotating synchronously in x or y directions (Carlsson et al. (1985) Opt. Lett. 10:53-55) to simultaneously scan an object with a point source of light and a point detector.

The above mentioned systems typically require several seconds to minutes to complete a scan of an object which prevents observation of the image by eye. The scan speeds of these devices are limited by both the mechanical scanning mechanisms used and the number of available photons for detection. These systems typically rely on photosensitive devices, such as photomultiplier tubes (PMTs) for detection and therefore must display the image as a raster scan on a monitor. This mode of imaging also precludes the direct observation of the image by eye and requires the need for complex electronics and computers for image generation.

Faster scanning rates have been achieved by using either an acousto-optic deflector (AOD) and a single rotating mirror (Draaijer and Houpt, U.S. Patent 4,863,226) or two AODs (Goldstein, U.S. Patent 4,802,748 (1989). These systems also require either photomultiplier tubes (PMT) or an image dissector tube (in the case of the latter system) as detectors. Although these systems can typically scan an object at rates of 30 scans per second, images of the object can not be directly viewed without the use of monitors, complex electronics, and computers.

The systems that use laser scanning mechanisms also require relatively large enclosures to house the scanning mechanisms, required optics, detectors and associated electronics. The size of these systems not only require additional workspace over and above the space required for a conventional microscope, but preclude the unobtrusive incorporation of these devices into the body of a conventional microscope instrument. Also, the design of these devices does not permit the use of their detectors for conventional modes of microscopy.

Baer (U.S. Patent 3,547,512 (1970)) describes a mechanically resonant structure that synchronously oscillates illumination and source slits in directions perpendicular to their long axes to confocally image a stationary object. In another approach, Baer used a spinning disc with pairs of diametrically opposed slits to effect illumination and imaging of an object. This invention has not been used for practical applications.

Koester (U.S. Patents: 4,170,398 and 4,241,257) developed a multifaceted scanning mirror system that used fixed slits to define the limits of illumination and detection of an object. This system does not provide direct viewing by human eye of the

image of an object; does not provide for the use of a laser light source; and does not provide for conventional microscopy.

An alternative method involves modifying an epi-illumination microscope such that, a single slit placed in the original specimen plane is moved along one axis to simultaneously scan a specimen and the face plate of a SIT camera (Lichtman, et al. (1989) The New Biol. 1:75-82). This approach requires considerable modification of a normal microscope and therefore is considered less desirable.

None propose direct visual human eye scanning of confocal microscopy observations while simultaneously providing direct or remote recording of the viewed object in the object plane.

In tandem scanning microscopy (TSM) an illuminated array of pinholes is imaged onto a specimen and the emitted light is detected by passing through a corresponding array of pinholes placed in a conjugate plane (accomplished with a rotating Nipkow disc) (Petran, et al. (1985) Scanning 7:97-108). Kino and Xiao (U.S. Patent 4,927,254) use a Nipkow disc for confocal microscopy in which corresponding pinholes are used for both illumination and detection. These systems are typically limited by the availability of photons and are more mechanically complex than the invention described herein.

Objects and Advantages

Accordingly several objects and advantages of my invention obtain and among them is the:

(a) provision of a scanning optical microscope instrument that gives a human operator an ability to select one of several optical paths each capable of providing a different mode of imaging that can be veiwed either sequentially or simultaneously;

(b) provision of a scanning optical microscope instrument that permits direct viewing by the human eye of images of a scanned object;

(c) provision of a scanning optical microscope instrument that permits easy conversion from a scanning optical microscope to a conventional microscope by conveniently shifting the scanning elements out of an optical path;

(d) provision of a scanning optical microscope instrument that can use a two-dimensional array detector to capture images of

an object from two or more optical paths either simultaneously or sequentially;

(e) provision of a scanning optical microscope instrument that permits multiple color-differentiated simultaneous imaging of an object for qualitative and quantitative analyses;

(f) provision of a scanning optical microscope instrument that can selectively use laser and conventional light sources;

(g) provision of a multiple purpose scanning optical microscope instrument that is relatively simple and easy to use;

(h) provision of a scanning optical microscope instrument that is relatively small, portable and compact with extended range of use and application.

Further objects and advantages are the provision of a multiple purpose scanning optical microscope that can be used as an effective and accurate analytical tool without the need for expensive electronics, computers and detectors; and which is economically advantageous to manufacture. Still further objects and advantages, including the provision of a single instrument with multiple capabilities at reasonable cost, will become apparent from a consideration of the ensuing descriptions and drawings.

In the Drawings

Fig. 1a is a schematic plan view of the preferred embodiment of this invention showing two optical paths in which the conventional optical path is utilized.

Fig. 1b is a schematic plan view of the preferred embodiment of this invention showing two optical paths, in which a confocal optical path is utilized by interdicting the conventional optical path with a scanning mirror.

Fig. 2 is a schematic plan view of an optical means of producing simultaneous two-color images of the object with the use of two light splitters.

Fig. 3 is a schematic plan view of an embodiment of the present invention described in Fig 1 utilizing two scanning mirrors.

Fig. 4 is a schematic plan view of an embodiment of the present invention in which scanning is accomplished along one axis with a rotating mirror and along a second axis with a coupled

illumination and detection pinhole.

Fig. 5 is a schematic plan view of an embodiment of this invention that permits visualization of three-dimensional images without the need for image processing with a computer.

Fig. 6 is a schematic plan view of an aperture optically defined by coupling a thin light absorbing film to a focusing optical system.

General Description

This invention concerns an optical microscope instrument with plural optical paths. The instrument is capable of viewing directly or by a scanning means, a specimen in the object plane. The microscope utilizes an upright or inverted configuration and the image may be viewed as transmitted, reflected or fluoresced light. The illumination sources used with this microscope are conventional sources such as tungsten, tungsten-halogen, mercury, or xenon arc lamps and unconventional sources such as lasers. The major novelty of the present optical system permits the presence of more than one optical path; each optical path being capable of altering the nature of the illumination such that selective imaging and selected physical properties of the light may be measured by viewing directly with the eye or by the use of electro-optical detector devices. The selection of the desired optical path to be used in the instrument is made by convenient mechanical motion whereby a scanning or beam-directing element interdicts one of the optical paths, thereby selecting an alternate established path. The light directed through the selected path is eventually brought to focus at a congruent image plane to permit the image to be viewed directly through an ocular and detected by an electro-optical detector system. The optical paths may be designed specifically for the viewing of confocal images with monochromatic or polychromatic radiation; for viewing the relationship between various phases of polarized radiation; for observing the phase relationships between differentially retarded waves due to circular dichroism; and the like.

This invention is an integrated self-contained assembly with defined multiple optical paths, with convenient interchange between the multiple optical paths and ocular with two-dimensional

detector-viewing of normal field and scan field images.

These features dramatize the novelty of this invention and the novelty of the resulting instrument accentuates an unobvious advance in microscopy.

One embodiment of this invention is a microscope with a conventional optical path and a single alternate path in which confocal viewing of fluoresced or reflected radiation from the sample can be accomplished. This embodiment will be used as an example to illustrate the nature of the invention.

This confocal viewing capability is accomplished by the interdiction of the normal optical path of the microscope with a unique rotating, flat double-sided mirror. This mirror, when moved into the normal optical path, directs the light along a novel selected light path. This selected light path permits confocal imaging of the object and reinserts the confocal images of the object into the conventional optical path of the microscope thus permitting concurrent direct ocular viewing of confocal images. This unit is compact, efficient and easy to use. The selection of an optical-pathway can be activated by the movement of a single button, rod or knob. All of the other microscope features including condenser, illuminator, objectives and other modes of contrast enhancement (i.e., dark field, phase contrast, etc.) are possible using either selected optical path. In all, a microscope instrument is made compactly available to extend analytical observation to scanned objects.

Specific Description of the Preferred Embodiment

This unique dual optical path scanning system is shown in a typical embodiment in Fig. 1a and Fig. 1b. Fig. 1a shows a rotating double-sided mirror 7, in a neutral position allowing light from object 9, to be directly focused along conventional light path 16, at image plane 17, thus allowing visualization of a magnified image of object 9, through occular 18, by eye 19, of a human observor. The placement and function of the elements 1, 2, 3, 4, 5, 6, 13, 14 and 20 in the microscope structure of the present invention will be better appreciated in the examination of Figure 1b.

In Fig. 1b rotating double-sided mirror 7, is repositioned by lever mechanism ~~around and through the pivot~~ 20, as shown,

to interdict conventional optical path 16. In this position another selected light path 2 originating from light source 1, which for this embodiment is shown as a laser, produces the light beam traversing path 2, that passes through beam-correcting and focusing optical element 3, and adjustable slit 4. The narrow cursor of light thus produced is reflected by light splitting element 5, to mirror 6, fixed at an angle to reflect light beam 2 onto movable, rotating double-side mirror 7. Light beam 2, is scanned over the back of objective lens 8 by mirror 7, and is thus focussed and deflected through an appropriate angle to illuminate a volume element within, or on, object 9.

In this description the object 9, is regarded as being labeled with a fluorescent dye. Emitted fluorescent light 10, from object 9, is collected by objective lens 8, and reflected back along the optical path defined by the rotating double-sided mirror 7, mirror 6, and light splitting element 5. The longer wave lenghts of emitted fluorescent light 10, pass through light splitting element 5, and through an adjustable detection slit 11. In this way only the fluorescent light 10, originating from the object 9 is transmitted to mirror 12. Fluorescent light 10, reflected by mirror 12, passes through relay lens system 13, and is reflected by mirrors 14, and 15, onto the opposite side of rotating double-sided mirror 7. The fluorescent light 10, is thereby directed to image plane 17, to which it has been focused by relay lens system 13. The image at this plane may be viewed by an eye of a human being 19, through lens system 18.

Although the embodiment of Figure 16 is described using the detection of fluorescent light emitted by the object, reflected light from the object may also be detected. Such an embodiment is realized by replacing light splitting element 5, with a mirror that transmits 50% and reflects 50% of reflected light from object 9, and the addition of other well known optical elements as needed for convenience and safety.

A variant to the embodiment in Fig. 1a and Fig. 1b would be to remove mirror 14, and position mirrors 6, 12, and 15, at appropriate selected angles to provide a means whereby light reflected from one side of rotating mirror 7, is directed to the opposite side of rotating mirror 7, with the result that an image is formed at image plane 17 of Fig. 1b with the use of

three rather than four mirrors. Such a variant within the scope of the present invention provides an economy in microscope manufacture and provides a variant perimeter usage of space.

Fig. 2 is schematic of a portion of the optical path described in Fig. 1b which includes the use of a second light splitter 5a, behind aperture 11a, which creates a virtual aperture 11b, that is reflected by mirror 12a. Virtual aperture 11b, is offset in object space from the original aperture 11a. Both apertures 11a, and 11b, are imaged at image plane 17, by relay lens system 13, and a single rotating mirror 7.

These images are focused on the surface of two-dimensional detector 21, by relay lens system 18, thus creating two separate simultaneous images 22a, and 22b, using one detector 21, and an appropriate monitor 22. The read-out leads 29 connect the detector 21 to the monitor 22 to transmit the images to the monitor 22. Each image 22a, and 22b, is locked in registry and spacially displaced from the other on the surface of detector 21 and on the screen of monitor 22. The wavelength of light which forms each image is determined by the transmission and reflection characteristics of light splitters 5 (Fig. 1a and Fig. 1b), and 5a (Fig. 2), in the optical path. This emphasizes the optical versatility of the described plural path microscope, adding the dimension of detector monitoring and displaying which can be recorded.

By this novel microscope construction a plurality of images of an object emitting a plurality of wavelenghts can be detected using a plurality of light splitters creating separate multiple images on the detector surface.

Fig. 3 shows an embodiment of the invention using two rotating mirrors 7a and 7b, which are single-sided mirrors. In this embodiment, the engagement of the alternate light path by moving scanning mirrors 7a and 7b, into the optical axis of the objective 8 permits selective and variable magnification and demagnification of the image of the object.

Magnification is accomplished by deflecting illumination light 1, over a relatively small angle with rotating mirror 7a, while deflecting emitted light 10, over a relatively large angle with rotating mirror 7b. The degree of magnification depends on the relative differences between the angle of deflection of mirrors 7a and 7b. Demagnification is accomplished by reversing

the above procedure. In both cases this image sizing factor is applied to the magnification factor produced by the other optical elements being used and significantly expands the versatility of the instrument.

Fig. 4 shows an embodiment of the invention using a rotating double-sided mirror 7, to scan along one axis, while the second axis is mechanically scanned using coupled illumination and detection pinholes 23. In this way confocal images can be detected rapidly by two-dimensional detector 21 using a single axis scanning mirror mechanism.

Fig. 5 shows the schematic of an embodiment of this invention which permits the use of two two-dimensional detectors 21a and 21b, to provide three-dimensional images of object 8, without the need for image processing. This chosen optical path includes the following additional elements not found in Fig. 1: (a) relay lens system 24; (b) rotating 50/50 light splitter 25, which is coupled to a Z-axis movement along the optical axis of objective 8; (c) two two-dimensional detectors 21a and 21b; and (d) monitors 26a and 26b.

In this embodiment 50/50 transmitting/reflecting light splitter 25, significantly displaces the reflected image while having little or no effect on the transmitted image. This displacement is linked to the movement along the Z-axis of objective 8. The two dimensional detectors 21a and 21b, capture images of object 9, that are extended along the Z-axis by the motion of objective 8. Each extended image is shifted slightly along the Z-axis of object 9, with respect to the other image. Detectors 21a and 21b, are read-out to two separate monitors 26a and 26b. In this way stereo-image pairs are displayed on monitors 26a and 26b and which can be viewed by polarized light differentiation.

Alternatively, monitors 26a and 26b, can be replaced with a single color monitor and each stero-image displayed in a different color to produce anaglyphs for stereoscopic viewing. Another mode of stereoscopic viewing involves displaying sequentially alternating flickering images resolved by synchronized ocular viewing.

Fig. 6 shows a creation of a novel aperture defined by placement of thin absorbing material 27, along and parallel to the optical axis of focusing optical system 13a. Absorbing material 27, creates a defined aperture by limiting the optical trans-

mission boundary for the range of displacement of the focal point of optical system 13a, along the optical axis. This mechanism permits only light emmanating from point 28 to be transmitted.

Additionally, a three-dimensional spatial filter 30 can be created by mounting a physical aperture orthogonal to the optical path, whereby both the physical aperture and the aperture created by material 27, have a common spatial center point or line.

Such a device could be used in the optical path described by Fig. 1, in which aperture 11 is replaced by either absorbing material 27, generated aperture, or a three-dimensional spatial filter. Instead of an aperture being defined by a single thin absorbing material 27, an aperture may be defined by an arrangement of a series of materials along and parallel to the desired optical pathway of optical system 13a. These thin absorbing materials are arranged such that only light originating from a certain point and traversing in an exactly defined direction can reach the image plane.

Having thus described my invention and a preferred embodiment thereof, those skilled in the art will appreciate variations, modifications and changes and such variations, modifications and changes are intended to be included herein limited only by the scope of my hereinafter appended claims.

Claims

-1-

A scanning microscope with a plurality of optical paths for producing an image of an object, with selected light sources and optical means for directing light from said sources to said object by way of a selected optical path for selected imaging as conventional and confocal imaging, including:

(a) scanning optical elements and means to move said optical elements into a position to direct said light to said object by way of said selected optical path;

(b) an aperture structure in said optical path;

(c) optical means to image said light from said object to a final image plane;

(d) optical means in said optical path for directing the light from said aperture structure to a final image plane;

(e) optical means for direct human visual observation of said final image plane, whereby the movement of said scanning elements into said position directs said light onto said object and engages said selected optical path resulting in selected modes of illumination and imaging, including confocal imaging, permitting direct eye observation of the image of said object at said final image plane.

-2-

A scanning microscope of Claim 1 in which the optical means for directing said light to said object includes:

(a) a light splitter to illuminate said object and transmit light coming from said object;

(b) one or more apertures of selected form and configuration and selectively fixed and variable whereby the light reaching the object and reaching the eye is limited by the selective use of said aperture;

(c) means to vary the opening of said variable of said apertures;

(d) and one or more scanning elements that selectively produce scanned images in XY, XZ, YZ and diagonal planes.

-3-

A scanning microscope of Claim 2 in which said final image plane is by optical means directed to a plurality of two-dimen-

registration media.

-4-

In the two-dimenstional registration media of Claim 3 wherein said media selectively includes photographic film and two-dimensional array detectors which selectively provide detection of monochromatic and polychromatic light from said object, and electronic means to process and display signals from said detectors including the use of a computer.

-5-

A scanning microscope of the Claim 3 in which a plurality of said light splitters which are wave length dependent are used in combination with said one or more apertures to create a plurality of images, real and virtual, of said apertures whereby each said image is of a different wave length, said images of said object being physically displayed on the surface of said two-dimensional detector by optical means.

-6-

The scanning microscope of Claim 5 which includes detection and processing means to selectively, simultaneously and sequentially detect and display multiple wavelength images of light from the object including the means to selectively obtain ratioing of the multiple wavelength images on a point by point, line by line and area by area basis.

-7-

A scanning optical apparatus of Claim 2 in which holographic filters are selectively substituted for said apertures.

-8-

A scanning microscope of Claim 2 in which said scanning elements comprise:

two mirrors and means to move said mirrors into a position to direct said light to said object; and

means imparting selected XY motion to one of said mirrors to scan said object while said second of said mirors is movable in controlled synchrony with said first mirror to produce proportional variations in the magnification and demagnification

of the image of said object.

-9-

A scanning optical apparatus of Claim 8 in which said magnification and demagnification of the image of the object is by optical means imaged onto a two-dimensional detector detecting relative displacement of said optical means.

-10-

A scanning microscope of Claim 8 in which one of the XY motions in one of said mirrors is coupled and synchronized to one of the XY motions of the second mirror, and the XY motion of said second mirror is coupled to a Z motion along the optical axis, obtaining a third dimension of information from the object by said two-dimensional detector.

-11-

A scanning microscope of Claim 1 in which confocal imaging is realized by scanning along one axis with said scanning optical elements and along the second axis by moving a coupled ensemble of illumination and detection apertures, which selectively include a pinhole of selected variation configurations, to maintain confocal conditions.

-12-

In the scanning microscope of Claim 3 and including two two-di- mensional array detectors and a 50/50 transmitting/reflecting mirror mounted on a single rotational axis; means to move said 50/50 mirror to displace the reflected image with respect to the transmittted image; said displacement being linked to the movement along the Z axis to produce stereo-images without the need for digital processing which stereo-images are viewed by observing two separate monitors simultaneously and mutually exclusively by the eyes of a human being.

-13-

A structure in the scanning microscope of Claim 3 comprised of thin, light-absorbing material defining an aperture along and parallel to the optical axis of said focussing optical system, said aperture establishing transmission boundary limits

for the range of displacement of a focal point along an optical axis of said focussing optical system.

-14-

In the combination of Claim 13 wherein separate similar apertures are mounted orthogonal to said optical axis of said focussing optical system, whereby said apertures have a common spatial center point or line which results in a three-dimensional aperture as three-dimensional spatial filter.

-15-

In the structure of Claim 13 wherein said light absorbing material provides a plurality of aperture structures each said structure defining a single separate aperture along and parallel to the optical axis of said focussing optical system.

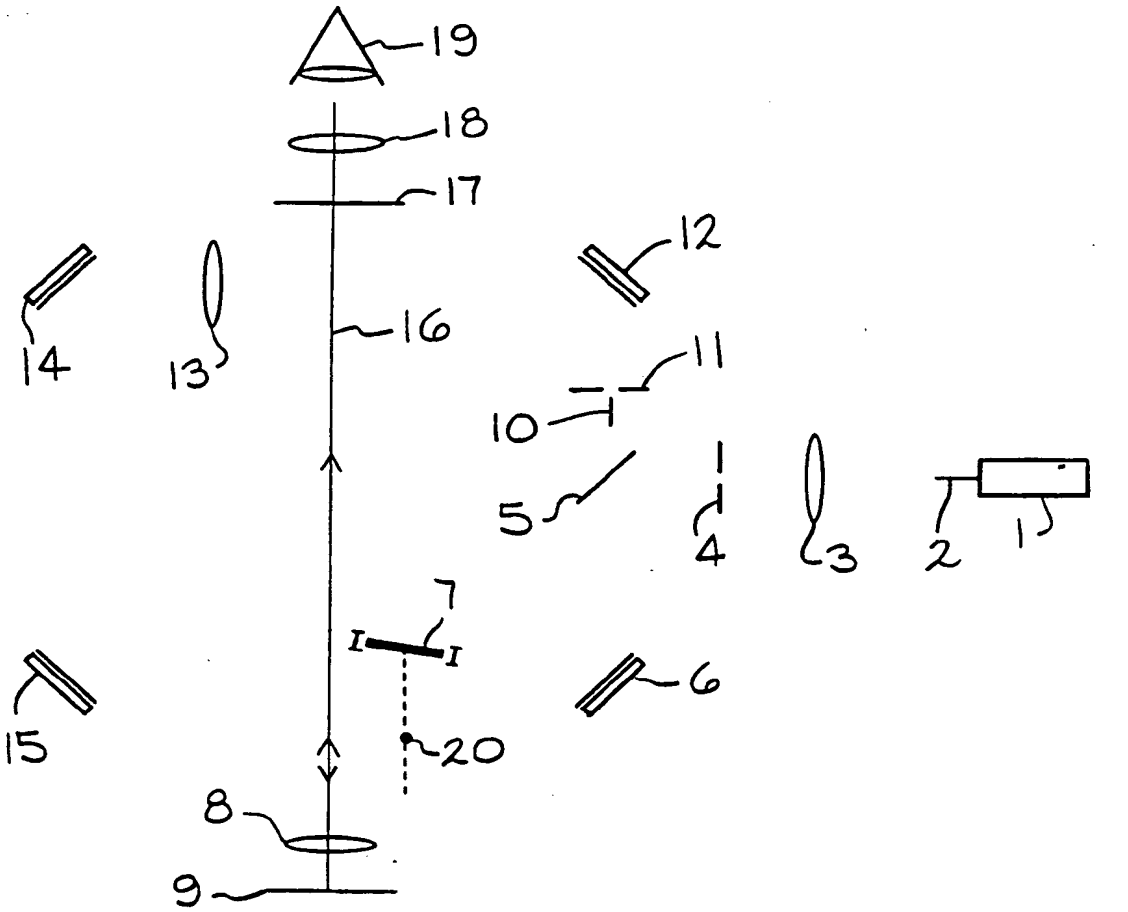

FIG. 1A

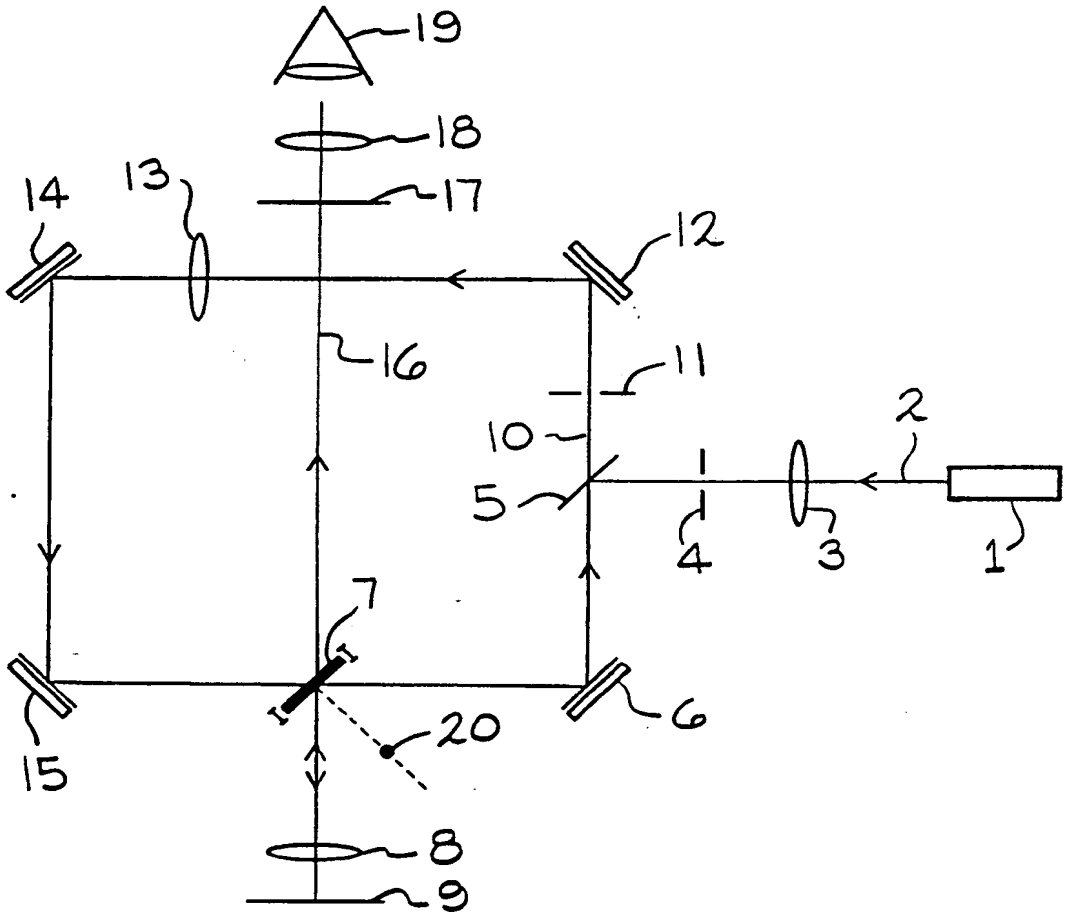

FIG. 1B

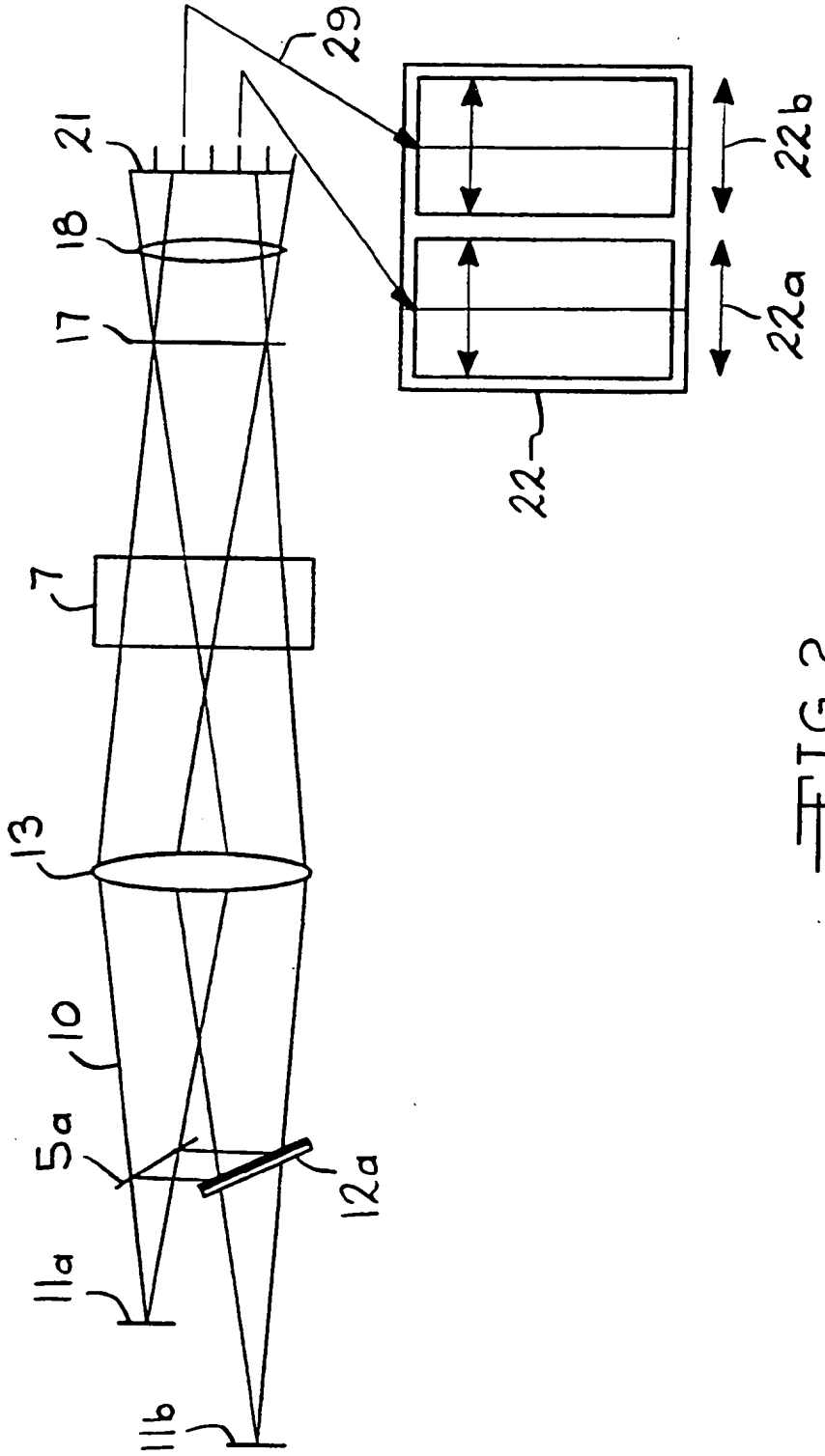

FIG. 2

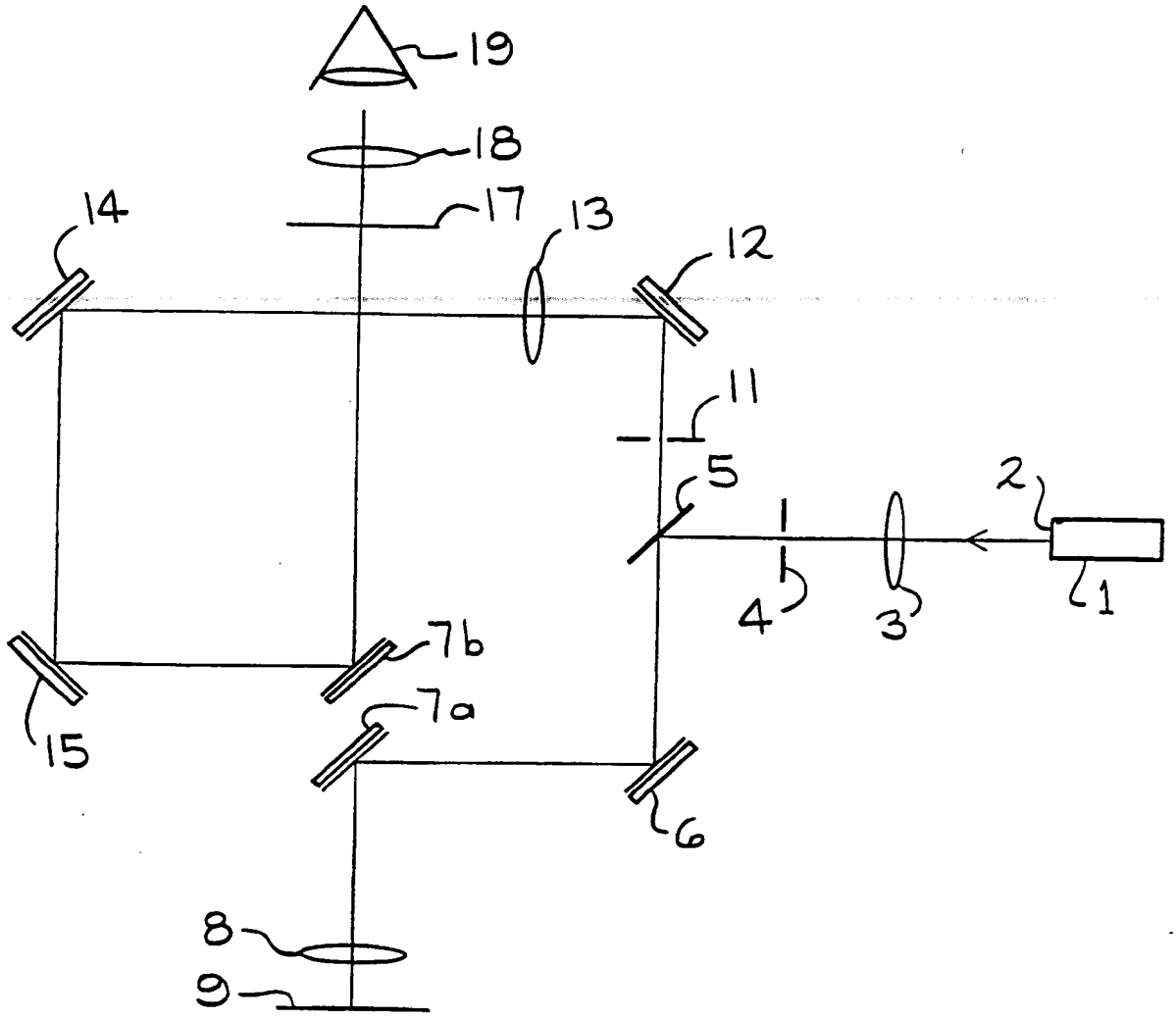

FIG. 3

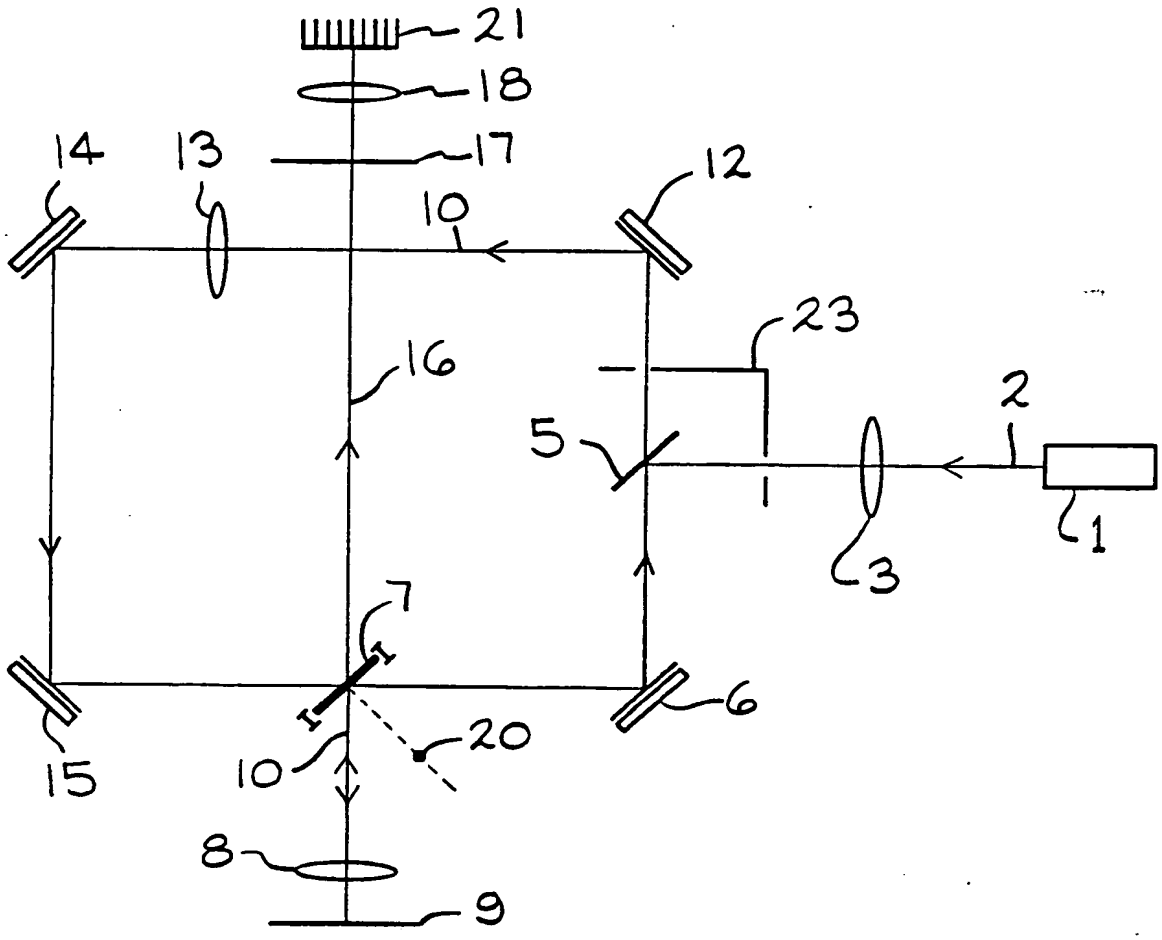

FIG. 4

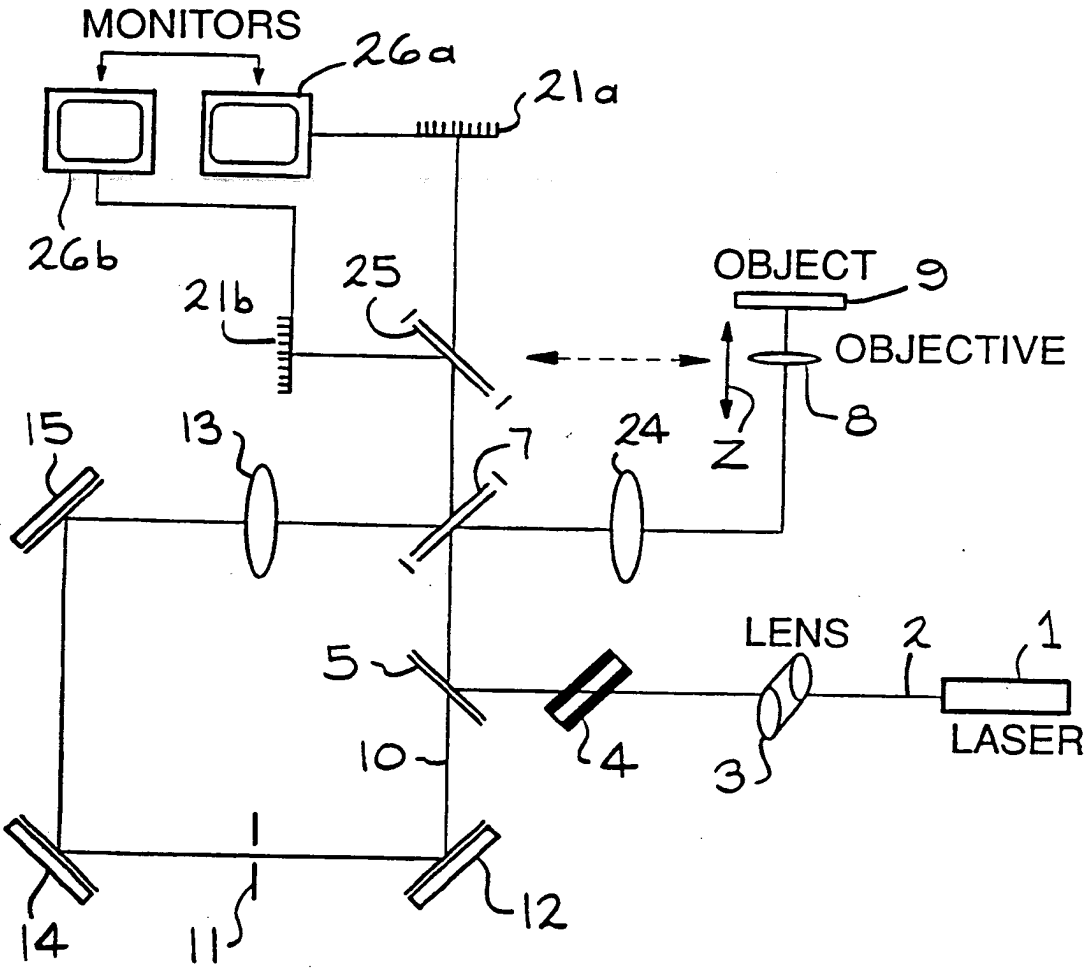
MONITORS
26a
21a
26b
21b
25
OBJECT
9
OBJECTIVE
8
Z
15
13
7
24
LENS
2
1
5
10
4
3
LASER
14
11
12

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/02376

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G02B 21/00; G02B 21/06; G02B 27/00
US.CL.: 350/507,523,527,274,276 SL; 250/201.3,225,234,216

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
350	507-509, 523, 527, 272-276SL, 448,6.3
150	201.2-201.5, 216, 225, 234-236

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US, A, 4,884,880 (LICHTMAN ET AL) 5 DECEMBER 1989 COLUMNS 6-9, FIGS. 1-2,4	1 2-15
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A	BRAKENHOFF ET AL, "CONFOCAL SCANNING LIGHT MICROSCOPY WITH HIGH APERTURE IMMERSION LENSES", VOL. 117, PT 2, PUBLISHED 1979 BY JOURNAL OF MICROSCOPY, SEE PAGES 219-232	1-15
A,E	US, A, 5,020,891 (LICHTMAN ET AL) 4 JUNE 1991 COLUMNS 3-6, FIGS 2,3	1-15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26 JUNE 1991	18 JUL 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	For B.Y. ARNOLD NGUYEN NGOC-HO INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (Rev.11-87)

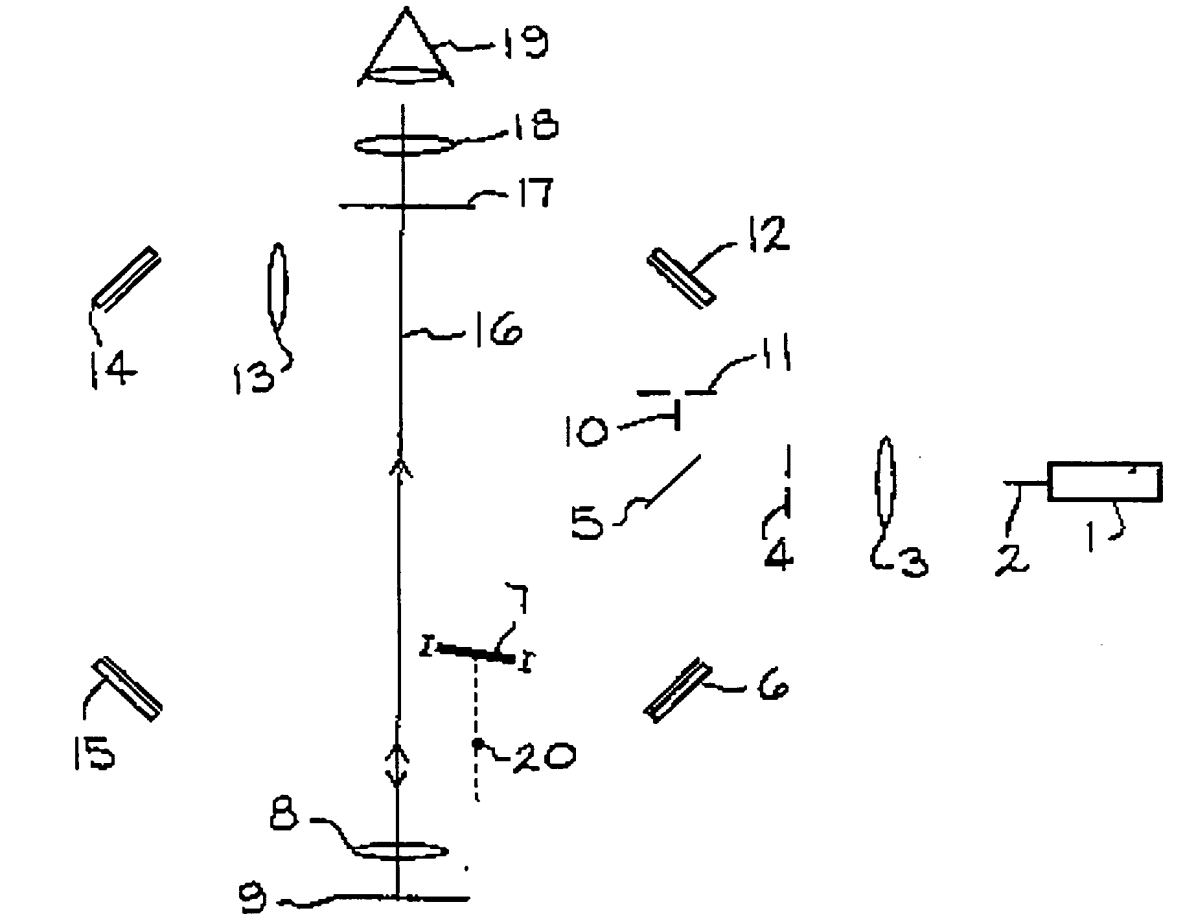

FIG. 1A

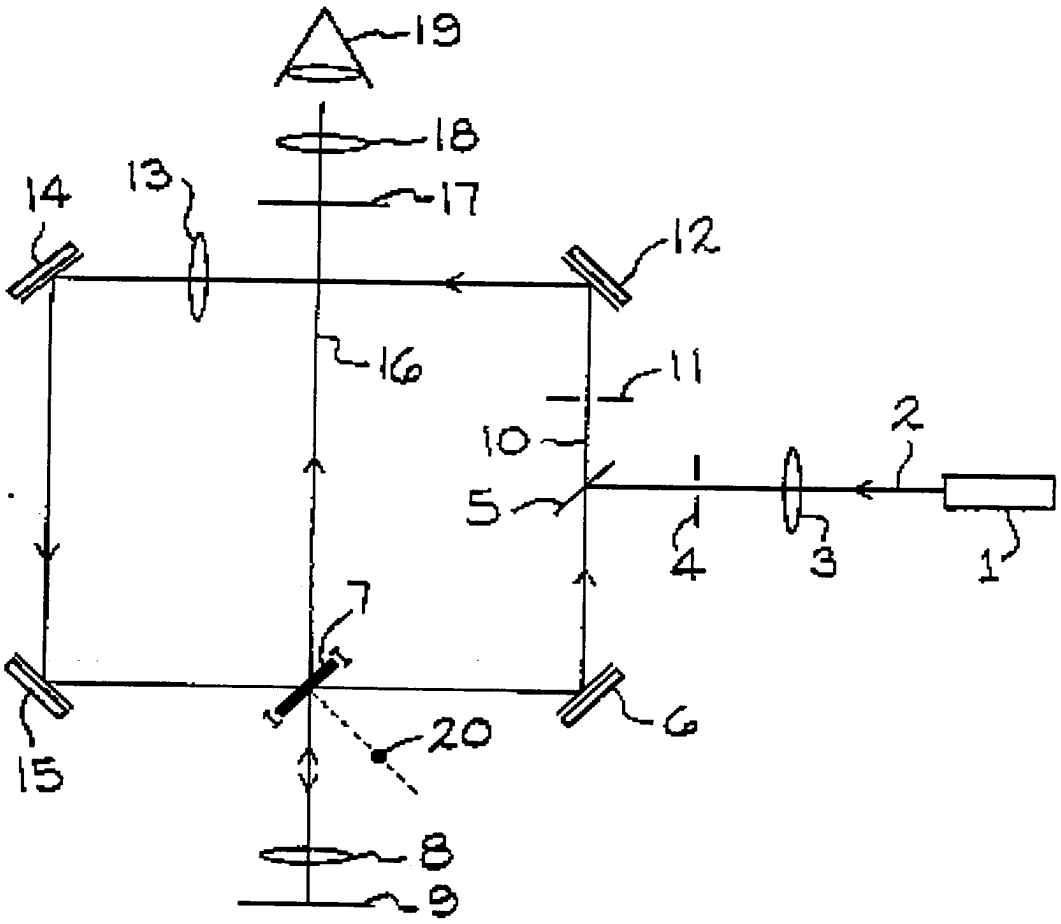

FIG.1B

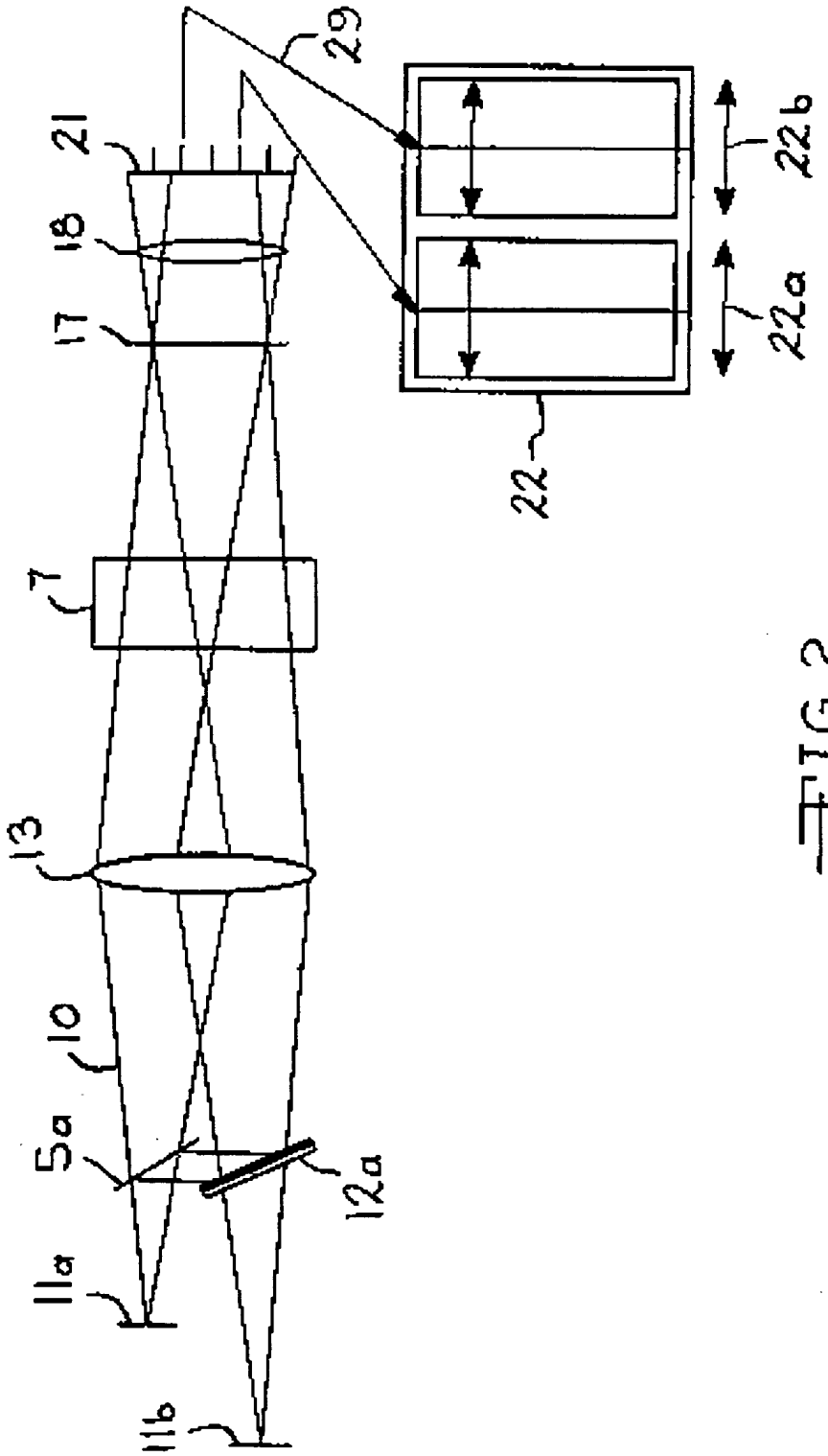

FIG. 2

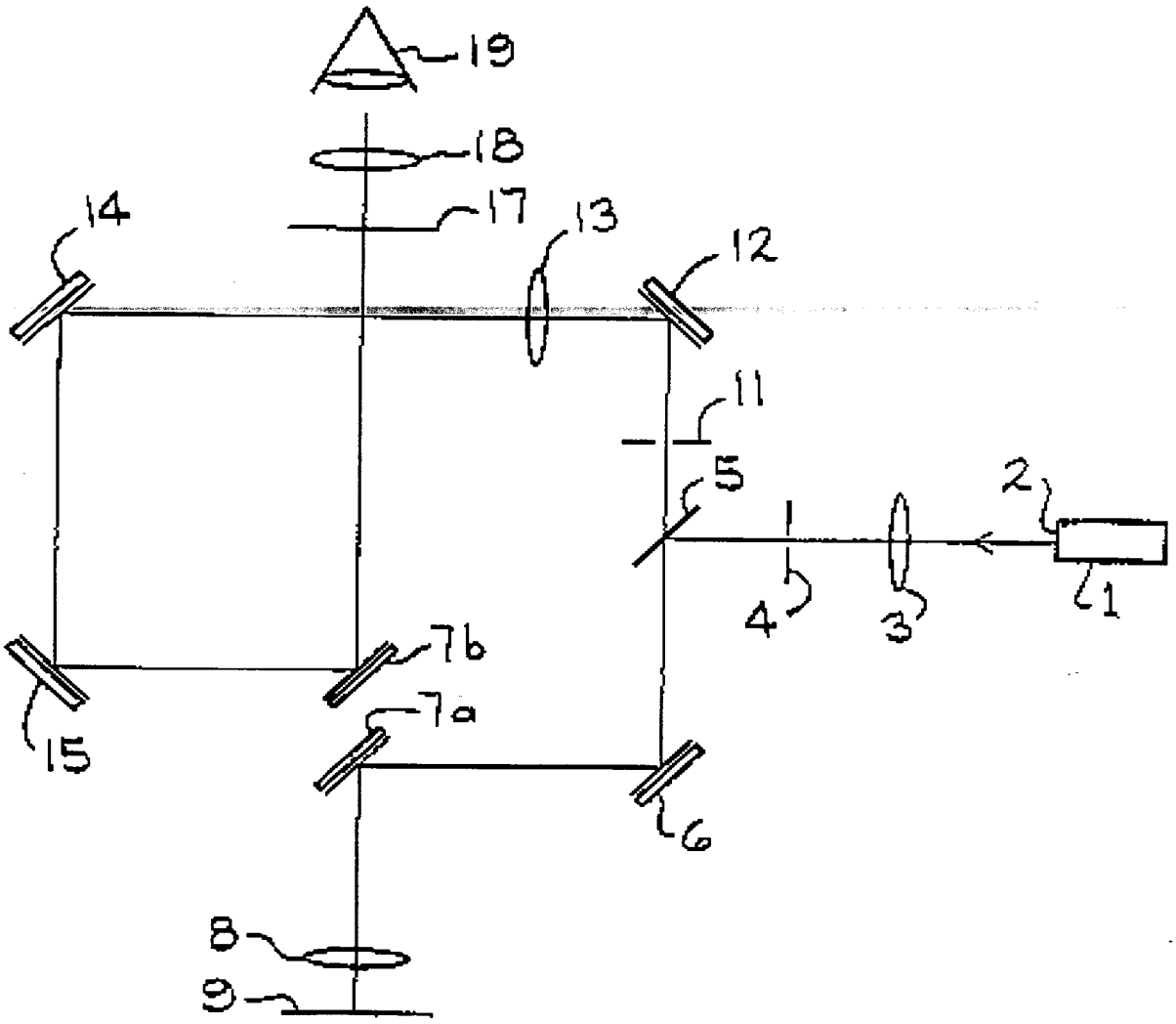

FIG. 3

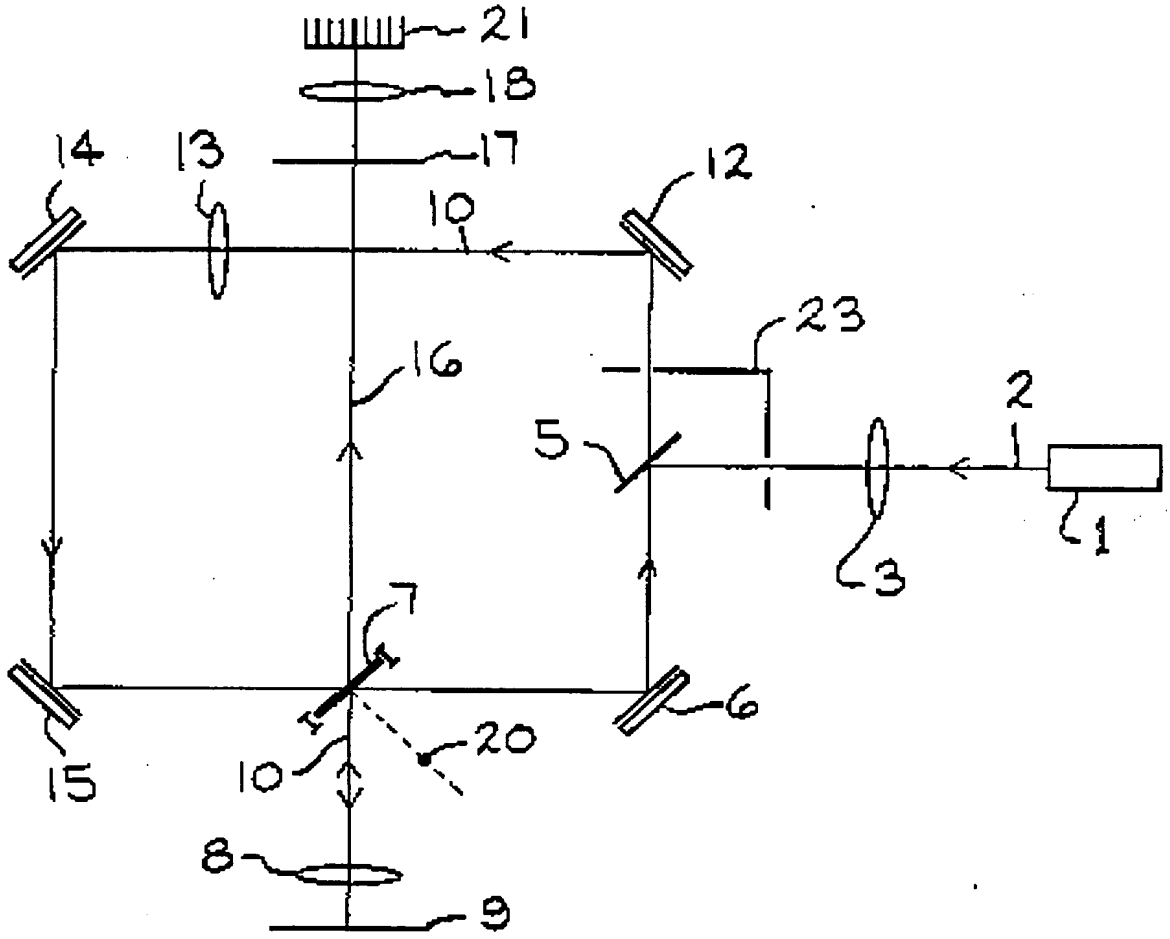

FIG. 4

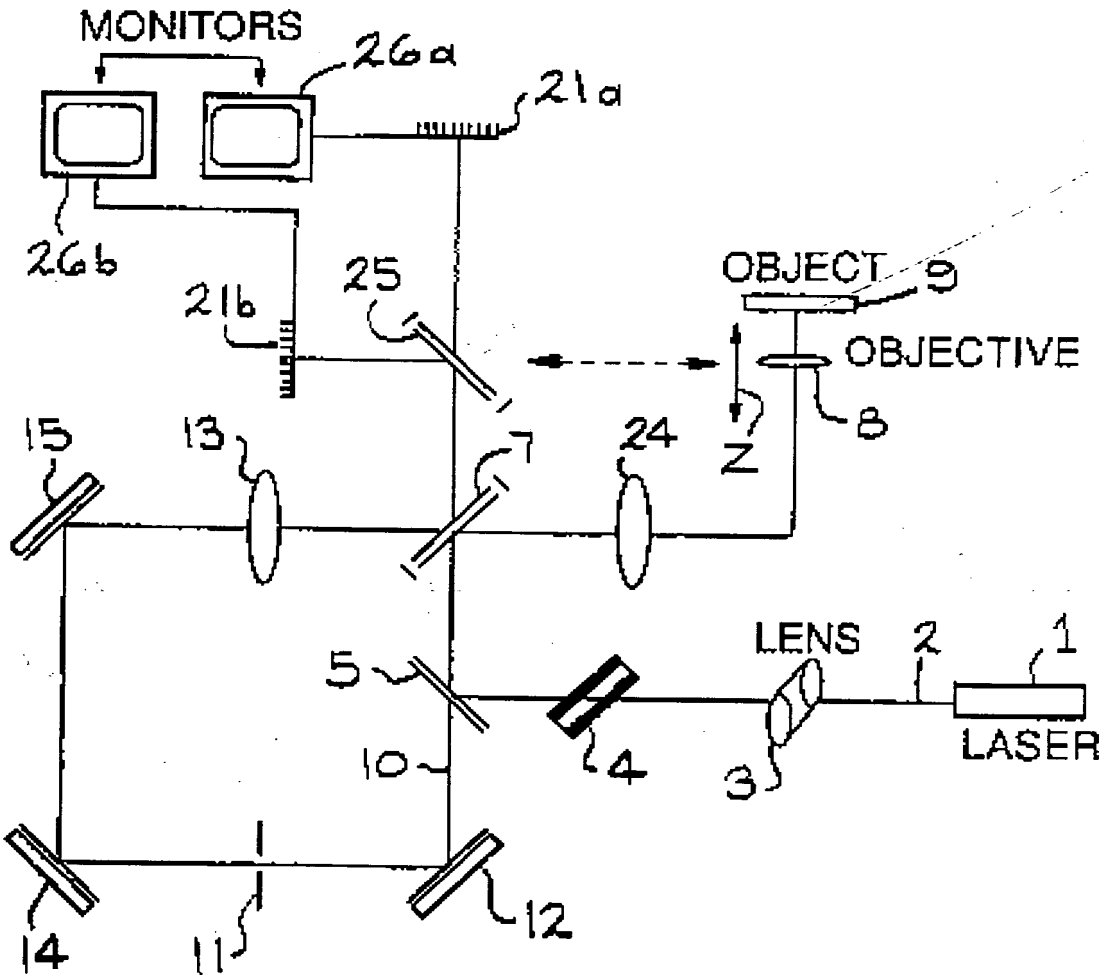

FIG. 5